# GENE FREQUENCIES OF HPA-1, -2 AND –3 AMONG MALAY AND CHINESE BLOOD DONORS IN HUSM

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| | |
|---|---|
| A | Adenine |
| C | Cytosine |
| CCI | Corrected count increment |
| CD | Cluster of differentiation |
| DNA | Deoxyrinonucleic acid |
| dNTP | Deoxynucleoside triphosphate |
| EDTA | Ethylene diaminetetra-acetic acid |
| ELISA | Enzyme-linked immunosorbent assay |
| FNAIT | Fetal and neonatal alloimmune thrombocytopenia |
| G | Guanine |
| GP | Glycoprotein |
| GVHD | Graft-versus-host disease |
| HGH | Human growth hormone |
| HLA | Human leucocyte antigen |
| HPA | Human platelet-specific antigens |
| HUGO | Human Genome Organization |
| HUSM | Hospital Universiti Sains Malaysia |
| ICH | Intracranial hemorrhage |
| IgG | Immunoglobulin G |
| IVIG | Intravenous immunoglobulin |
| IPD | Immuno Polymorphism Database |

| | |
|---|---|
| ISBT | International Society of Blood Transfusion |
| LCR | Ligase chain reaction |
| MAIPA | Monoclonal antibody-specific immobilisation of platelet antibodies |
| $MgCl_2$ | Magnesium chloride |
| MPHA | Mixed passive haemagglutination |
| NCBI | National Center for Biotechnology Information |
| OLA | Oligonucleotide ligation assay |
| PCR | Polymerase chain reaction |
| PCR-ASO | Polymerase chain reaction-allele specific oligonucleotides |
| PCR–SSP | Polymerase chain reaction using sequence-specific primers |
| PHFA | Preferential homoduplex formation assay |
| PIFT | Platelet immunofluorescence test |
| PNC | Platelet Nomenclature Committee |
| PTP | Post transfusion purpura |
| RFLP | Restriction fragment length polymorphism |
| SNP | Single nucleotide polymorphism |
| SPSS | Statistical Package for Social Sciences |
| SSCP | Single-strand conformation polymorphism analysis |
| SSO | Sequence-specific oligonucleotides |
| T | Thymine |
| UV | Ultraviolet |

7 ABSTRAK


**FREKUESI GEN HPA-1, -2 DAN -3 DI KALANGAN PENDERMA DARAH MELAYU DAN CINA DI HUSM**

'Human platelet-specific antigens' (HPA) terdapat pada permukaan membran glikoprotein platelet. Antigen-antigen ini adalah amat imunogenik dan menjadi punca kepada beberapa penyakit alloimmun trombositopenia seperti penyakit neonatal alloimmun trombositopenia, purpura selepas transfusi dan masalah refraktori kepada transfusi platelet. Pengecaman genotip HPA adalah penting kerana prevalen HPA berbeza mengikut populasi.

Objektif kajian ini adalah untuk menentukan frekuensi gen dan genotip HPA-1, -2 dan -3 di kalangan penderma darah Melayu dan Cina di Unit Perubatan Transfusi, Hospital Universiti Sains Malaysia. Kami juga ingin melihat perbandingan frekuensi di antara dua kaum ini.

Kajian ini telah dijalankan pada Ogos 2006 sehingga Julai 2007 dan telah melibatkan 117 penderma darah Melayu dan 103 penderma darah Cina. Sampel darah telah diambil daripada setiap subjek. DNA telah diasingkan menggunakan kit GENE✓ALL™. Pengecaman genotip HPA telah dilakukan menggunakan teknik 'allele-specific oligonucleotide polymerase chain reaction'. Keputusan kajian dianalisa menggunakan program SPSS versi 12.

Frekuensi alel bagi penderma darah Melayu adalah 100.0% dan 8.7% bagi HPA-1a dan 1-b; 99.1% dan 15.9% bagi HPA-2a dan -2b; 80.7% dan 78.9% bagi HPA-3a dan -3b. Bagi penderma darah Cina pula, frekuensinya adalah 100% dan 2.0% bagi HPA-1a dan -1b; 100% dan 4.9% bagi HPA-2a dan -2b; 80.0% dan 66.0% bagi HPA-3a dan -3b.

Bagi keputusan genotip HPA-1, 91.3% daripada penderma darah Melayu adalah homozigos untuk HPA-1a/a dan 8.7% heterozigos untuk HPA 1a/b. Bagi penderma darah Cina, 98.0% adalah homozigos untuk HPA-1a/a dan 2.0% adalah heterozigos untuk HPA 1a/b. Tiada sampel yang homozigos untuk HPA-1b/b. Bagi genotip HPA-2, 84.1% penderma darah Melayu adalah homozigos untuk HPA-2a/a, 15.0% adalah heterozigos untuk HPA 2a/b dan 0.9% homozigos untuk HPA-2b/b. Bagi penderma darah Cina, 95.1% adalah homozigos untuk HPA-2a/a dan 4.9% adalah heterozigos untuk HPA-2a/b. Tiada penderma darah Cina yang homozigos untuk HPA-1b/b. Bagi genotip HPA-3, 21.1% penderma darah Melayu adalah homozigos untuk HPA-3a/a, 59.6% heterozigos untuk HPA-3a/b dan 19.3% adalah homozigos untuk genotip HPA-3b/b. Bagi penderma darah Cina, 34.0% adalah homozigos untuk HPA-3a/a, 46.0% heterozigos untuk HPA-3a/b dan 20.0% adalah homozigos untuk HPA-3b/b.

Kajian ini telah menunjukkan perbezaan frekuensi gen dan genotip di antara penderma Melayu and Cina. Data ini akan menggunakan data ini untuk penghasilan HPA 'HPA-typed' di HUSM. Kajian ini akan dilanjutkan pada masa akan dating untuk HPA lain di dalam system ini.

## 8 ABSTRACT


### GENE FREQUENCIES OF HPA-1, -2 AND –3 AMONG MALAY AND CHINESE BLOOD DONORS IN HUSM

Human platelet-specific antigens are found on the platelet membrane glycoproteins. These antigens are highly immunogenic and are implicated in alloimmune thrombocytopenias such as in fetal and neonatal alloimmune thrombocytopenia, post transfusion purpura and refractoriness to platelets transfusion. HPA genotyping is important because the prevalence of HPA differs among various populations.

The objective of this study was to determine the HPA-1, -2 and -3 genotypes and gene frequencies in Malay and Chinese blood donors in Transfusion Medicine Unit, Hospital Universiti Sains Malaysia (HUSM). We would also like to compare the distribution of HPA between those two ethnic groups.

A cross-sectional study was done in the Transfusion Medicine Unit, HUSM from August 2006 till July 2007. Blood specimens were collected from 117 of Malay and 103 of Chinese blood donors. DNA was isolated using GENE✓ALL™ Blood SV mini kit. HPA-1, -2 and -3 genotyping were determined by using the allele-specific oligonucleotide polymerase chain reaction (PCR-ASO) method. The results were analysed by SPSS version 12.

The allele frequencies obtained for the Malay blood donors were 100% and 8.7% for HPA-1a and -1b; 99.1% and 15.9% for HPA-2a and -2b; 80.7% and 78.9% for HPA-3a and -3b. For the Chinese blood donors, the results were 100% and 2.0% for HPA1a and -1b; 100% and 4.9% for HPA-2a and -2b; 80.0% and 66.0% for HPA-3a and -3b.

As for HPA-1 genotype results, 91.3% of the Malay blood donors were homozygous for HPA-1a/a and 8.7% were found heterozygous for HPA 1a/b. In the Chinese blood donors, 98.0% were homozygous for HPA-1a/a and 2.0% were found heterozygous for HPA 1a/b. None of the samples were homozygous for HPA-1b/b. Concerning the HPA-2 genotype, 84.1% of the Malay blood donors were homozygous for HPA-2a/a, 15.0% were heterozygous for HPA 2a/b and 0.9% were homozygous for HPA-2b/b. In the Chinese blood donors, 95.1% were homozygous for HPA-2a/a and 4.9% were heterozygous for HPA-2a/b. None of the Chinese blood donors were homozygous for HPA-2b/b. Regarding the HPA-3 genotype, 21.1% of the Malay blood donors were homozygous for HPA-3a/a, 59.6% were heterozygous for HPA-3a/b and 19.3% were homozygous for HPA-3b/b genotype. In the Chinese blood donors, 34.0% were homozygous for HPA-3a/a, 46.0% were heterozygous for HPA-3a/b and 20.0% were homozygous for HPA-3b/b.

This study revealed the differences between the Chinese and Malay HPA genotypes. These data will be used to develop an HPA-typed platelet donor registry in our hospital. Other HPAs in this system will be typed in the future,

# Chapter 1
# Introduction

## 1.0 INTRODUCTION

Human platelet-specific antigens (HPA) are found on the four major platelet membrane glycoproteins (GP): GPIa, GPIb, GPIIb and GP IIIa (Figure 1.1). These antigens are highly immunogenic and become the target of platelet alloantibodies. Platelet alloantibodies are formed due to sensitization during pregnancy or previous random platelet transfusion. These alloantibodies are involved in the pathogenesis alloimmune thrombocytopenias as in fetal and neonatal alloimmune thrombocytopenia, post transfusion purpura and refractoriness to platelet transfusion (Metcalfe et al., 2003).

HPA systems consist of more than 12 bi-allelic antigen polymorphisms which are grouped in six biallelic systems (HPA-1, -2, -3, -4, -5, -15) (Metcalfe et al., 2003). The notations used are "a" for the common alleles and "b" for the rare alleles. In general, the molecular basis of this polymorphism was identified as single base changes.

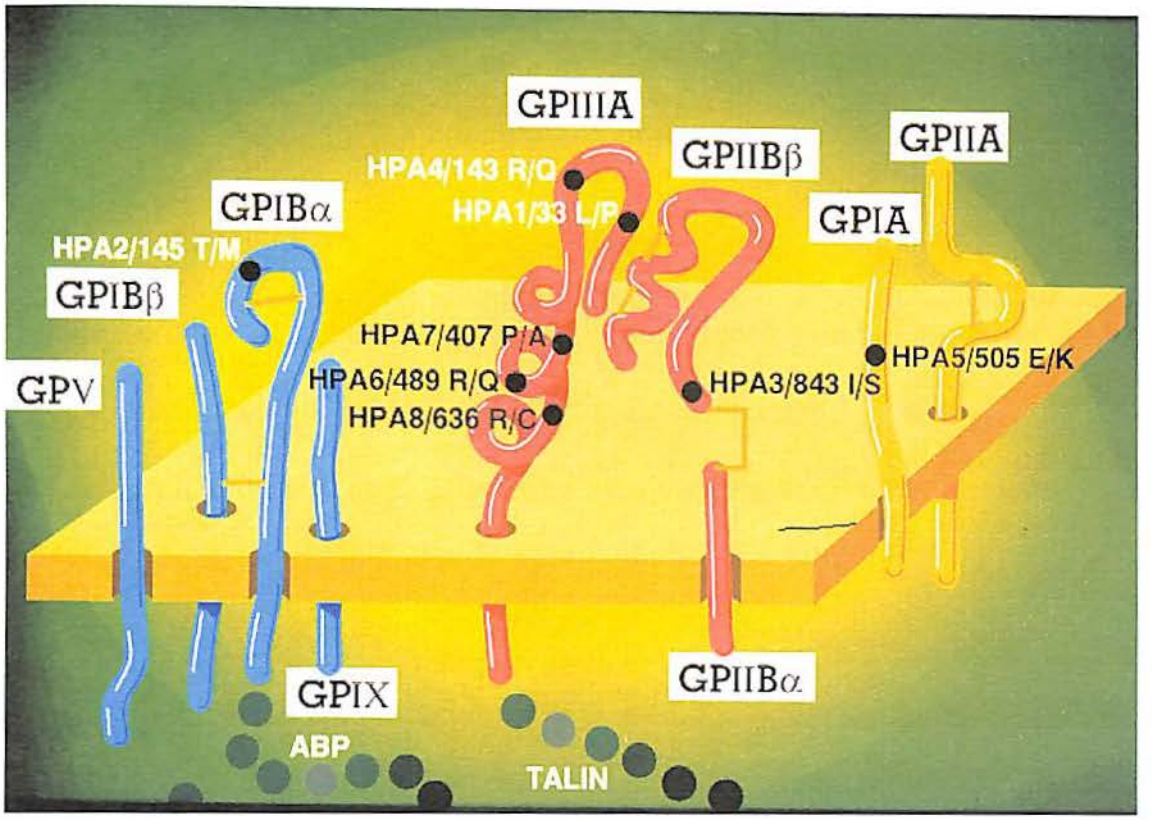


**Figure 1.1 Platelet** membrane glycoproteins (GP) and human platelet-specific antigens. (Adapted from Platelet Immunology Workshop 2005)

In clinical practice, all human platelet antigens are important in the pathogenesis of alloimmune thrombocytopenias. HPA-1 has also been implicated in the pathogenesis of thrombotic disorders such as in acute coronary syndromes (Wagner et al., 1998) and ischaemic stroke (Weiss et al., 1996). Thus, rapid and accurate platelet typing is essential in the diagnosis and management of these patients.

Population studies have shown that gene frequencies of HPA vary between different ethnic groups (Kekomaki et el., 1995, Kim et al., 1995, Bennett et al., 2002, Bianchi et al., 2005). To date, only few studies have been published regarding the prevalence of HPA in the Malaysian population.

Different methods in determining human platelet-specific has been used including immunophenotyping, enzyme-linked immunosorbent assay (ELISA), haemagglutination assay and polymerase chain reaction (PCR) methods. Newer technologies such as DNA microarrays have also been applied in platelet genotyping.

In serological methods, human sera are needed. Fresh panels are required for optimal results and antibodies to antigens weakly expressed e.g. HPA-5. Good quality typing sera are scarce due to the low immunogenicity of HPA (Use of PCR in laboratory testing, 2002). Because of all these problems, it is now more common to perform genotyping of platelet antigens by using PCR method.

In this study, gene frequencies of for HPA-1, -2 and -3 in blood donors will be determined using polymerase chain reaction (PCR) - allele specific oligonucleotides (ASO) method.

PCR is in-vitro amplification of specific DNA sequences by simultaneous primer extension of complementary strands. By using this method, numerous copies of small fragments of genetic material can be produced to facilitate the analysis of genetic material (Use of PCR in laboratory testing, 2002).

Typing by PCR is simple, accurate and reliable. It is recommended by Ministry of Health in Malaysia as the method of choice in many fields including transplant programme, identification of post transfusion purpura, zygosity testing, antenatal diagnosis, investigation of thrombocytopenic patients and many others (Use of PCR in laboratory testing, 2002).

## 1.1 RATIONALE OF STUDY

In view of the clinical importance of HPA, we would like to study the distribution of the HPA genes in our population. The knowledge and understanding of HPA gene frequencies will assist us to predict the risk of platelet alloimmunization in our population.

The results obtained for this study will be used to establish an HPA-typed donor registry for platelet donors and will be benefitted in future for the management of platelet transfusion in this hospital. This in turn could provide a more effective platelet transfusion therapy in conditions associated with platelet alloimmunization. The role of HPA in several diseases and transplantation has been investigated and will be helpful to extend in future clinical practice.

# Chapter 2
# Literature Review

# 2.0 LITERATURE REVIEW

## 2.1 Platelet alloantigens

Platelet alloantigens are defined by alloantibodies directed against genetically determined molecular variations of proteins or carbohydrates on the platelet membrane (Santoso S, 2003). There are two types of clinically significant platelet alloantigens namely Type I and Type II. Type I alloantigens are shared with other blood cells and tissues. Whereas the Type II alloantigens are more specific to platelets and are called platelet-specific alloantigens. Platelet-specific alloantigens are polymorphic epitopes of platelet membrane glycoproteins and become the targets of platelet alloantibodies (Koutsogianni P, 2004).

## 2.2 Human platelet-specific alloantigen system

Human platelet antigens (HPA) were first described in the late 1950s and are inherited by autosomal co dominance (Mohanty et al., 2004). It was in 1990, the Working Party on Platelet Serology, decided to formulate a new HPA nomenclature system. According to this system, the platelet-specific antigen systems will be called HPA for Human Platelet Antigens. The different antigen systems will be numbered according to the date of publication and the allelic antigens will be designated alphabetically, according to their frequency in the population. The inclusion of any new HPA systems will need the approval of the working party.

In 2003, the Platelet Nomenclature Committee (PNC) has been created as collaboration between the International Society of Blood Transfusion (ISBT) platelet working party and the International Society on Thrombosis and Haemostasis (ISTH) scientific subcommittee on platelet immunology (Metcalfe et al., 2003). These committee will be the guardian of the HPA nomenclature system and has added some new rules to the HPA system. The additional rules were as follow:

1. A platelet-specific alloantigen is called HPA when its molecular basis has been defined.
2. A .w. designation is added after the antigen name if an alloantibody against the antithetical antigen has not been reported.
3. New antigens will only be included in the HPA nomenclature table when approval from the PNC has been obtained.

To date, twenty-four platelet-immune sera have defined specific alloantigens. Twelve of them are grouped in six biallelic systems (HPA-1,-2,-3,-4,-5,-15). Three linked nomenclature tables will be maintained. The first table as shown in Table 2.1 showed the HPA antigens in chronological order, the original names, glycoprotein name and cluster of differentiation (CD) designation. The second table will contain the antigens arranged according to their genetic basis. This table will show the details on the nucleotide and amino acid substitutions (Table 2.2). The third table lists all the alleles which have been defined by DNA sequencing (Table 2.3).

**Table 2.1** Human platelet antigens (HPAs)

| System | Antigen | Original names | Glycoprotein | CD |
|---|---|---|---|---|
| HPA-1 | HPA-1a<br>HPA-1b | $Zw^a$, $Pl^{A1}$<br>$Zw^b$, $Pl^{A2}$ | GPIIIa | CD61 |
| HPA-2 | HPA-2a<br>HPA-2b | $Ko^b$<br>$Ko^a$, $Sib^a$ | GPIb*alpha* | CD42b |
| HPA-3 | HPA-3a<br>HPA-3b | $Bak^a$, $Lek^a$<br>$Bak^b$ | GPIIb | CD41 |
| HPA-4 | HPA-4a<br>HPA-4b | $Yuk^b$, $Pen^a$<br>$Yuk^a$, $Pen^b$ | GPIIIa | CD61 |
| HPA-5 | HPA-5a<br>HPA-5b | $Br^b$, $Zav^b$<br>$Br^a$, $Zav^a$, $Hc^a$ | GPIa | CD49b |
| | HPA-6bw | $Ca^a$, $Tu^a$ | GPIIIa | CD61 |
| | HPA-7bw | $Mo^a$ | GPIIIa | CD61 |
| | HPA-8bw | $Sr^a$ | GPIIIa | CD61 |
| | HPA-9bw | $Max^a$ | GPIIb | CD41 |
| | HPA10bw | $La^a$ | GPIIIa | CD61 |
| | HPA11bw | $Gro^a$ | GPIIIa | CD61 |
| | HPA12bw | $Iy^a$ | GPIb*beta* | CD42c |
| | HPA13bw | $Sit^a$ | GPIa | CD49b |
| | HPA14bw | $Oe^a$ | GPIIIa | CD61 |
| HPA-15 | HPA-15a<br>HPA-15b | $Gov^b$<br>$Gov^a$ | CD109 | CD109 |
| | HPA-16bw | $Duv^a$ | GPIIIa | CD61 |

Adapted from the Immuno Polymorphism Database (IPD) (www.ebi.ac.uk/ipd)